Transgenerational epigenetic inheritance: from biology to society—Summary Latsis Symposium Aug 28–30, 2017, Zürich, Switzerland

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Abstract: In biology, inheritance is a process that ensures the transfer of features and traits from parent to offspring. The most classic view of parental inheritance is that it is genetic and is embedded in genes contained in the genome in germ cells. However, genetic inheritance is now known to contribute to only a part of what an individual can transmit to its progeny. Thus, further to innate traits that each individual receives from its parents, acquired traits, which are traits acquired upon exposure to environmental factors or personal experiences, can also be inherited. This form of inheritance is not encoded in the sequence of genes but is mediated by mechanisms and processes elicited by the environment that modify the activity of the genome persistently across generations. Because it is not encoded in DNA sequences, it is called epigenetic or non-genetic. These mechanisms establish a link between the genome and the environment. They relate to the extremely important question of nature versus nurture namely, how much our own make-up is genetically or epigenetically determined, a question that remains unresolved. In August 2017, an international symposium was organized in Zürich, Switzerland to address the question of epigenetic inheritance. The Latsis symposium 2017 on "Transgenerational epigenetic inheritance: from biology to society" held at the ETH Zürich gathered international leaders in the field and focused on major questions and current challenges raised by the concept of epigenetic inheritance. The symposium was one of the first fully dedicated to the theme of epigenetic inheritance and covered scientific aspects from invertebrates to humans, and from behavior to metabolism in humans and animal models, mental health and epidemiology, bioinformatics and ethics. The symposium lasted 2.5 days and was attended by about 150 people from different countries. The program was structured in sessions of 3–3 h 30 min each (total of five sessions) including invited talks and short oral presentations. This summary provides an overview of the speakers' presentations and focuses on four major topics: (i) evidence and challenges for epigenetic inheritance in humans, (ii) new insight and major questions raised by work in animal models, (iii) methodologies in epigenetics and (iv) evolution, societal impact and broader considerations.

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Research Highlight

RESEARCH HIGHLIGHT

Transgenerational epigenetic inheritance: from biology to society—Summary Latsis Symposium Aug 28–30, 2017, Zürich, Switzerland

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Evidence and Challenges for Epigenetic Inheritance in Humans

The first session focused on clinical epigenetics, and was started by Marcus Pembrey (University College London) with a

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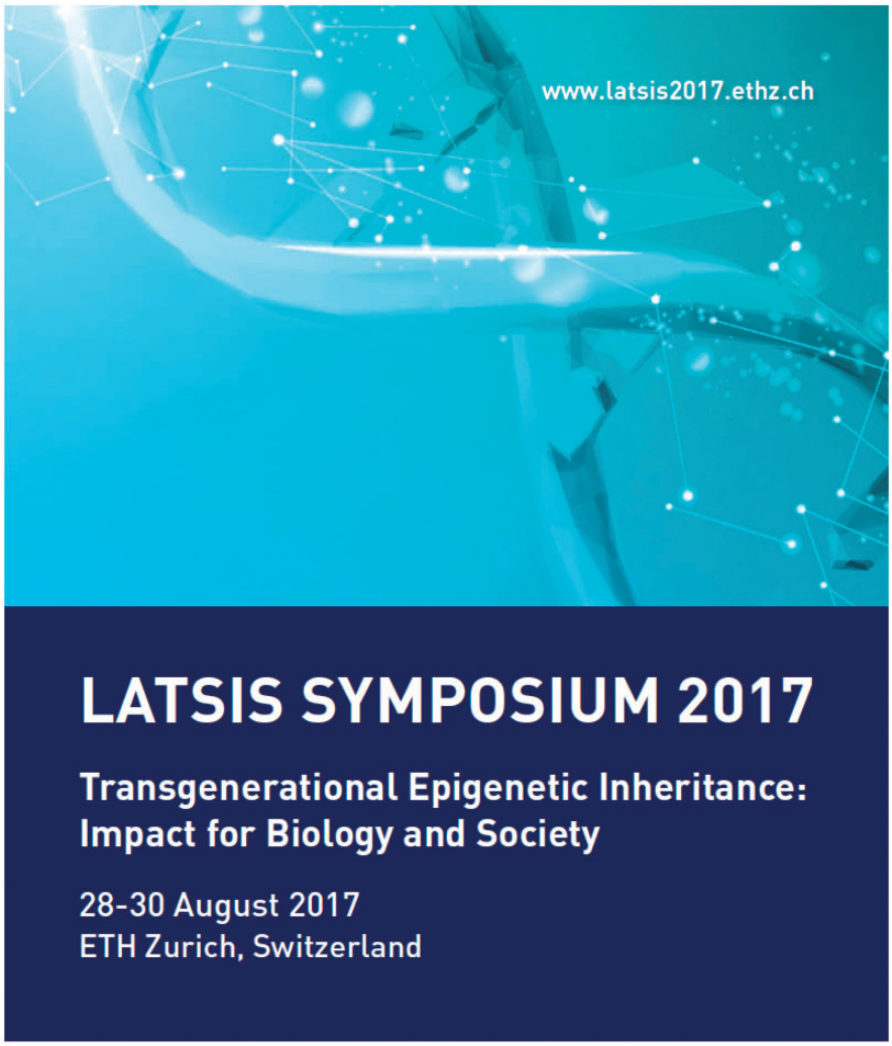

review of his long-standing work in epigenetic epidemiology, in particular related to food deprivation and smoking. He presented new DNA methylation (DNAme) results shedding light on possible mechanisms of transmission. His talk touched a number of issues critical to the field, from the identification of exposure-sensitive developmental periods to the interpretation of underlying gene and phenotypic differences between the first generations, and the discrepancy between symptoms transmission and changes in DNAme. Markus Pembrey presented recent work on the Avon Longitudinal Study of Parents and Children (ALSPAC), showing that autism risk is higher in granddaughters if the maternal grandmother has smoked during pregnancy. Autism is one of many devastating neuropsychiatric disorders in which exposure of past generations is likely to play an important role in disease etiology. This is a vivid example demonstrating the importance of research in the field of epigenetic inheritance and the high stakes regarding implications for patients and families. Another question addressed by Pembrey was that of the sensitive period during which the germline is particularly responsive to environmental factors. He reviewed the seminal work he did with Lars Olov Bygren on the impact of food availability on health and mortality of grandoffspring in the Överkalix cohort where the concept of sensitive period and transgenerational programming was first introduced. He pointed out that the effects of scarce food supply is difficult to disentangle from the effects of stress resulting from unfavorable living conditions and famine. This work highlights that early life experiences of parents and ancestors contribute to population developmental variation beyond that due to social transmission.

Adelheid Soubry (University Leuven) then discussed different studies on the impact of paternal diet and exposure to chemicals on sperm and offspring. She presented an update on several large human cohort studies and new work on the impact of organophosphate (OP) flame retardants. Measuring OP levels in men and correlating it to DNAme in sperm revealed

OP-related changes in DNAme at imprinted genes. An interesting finding was that "cocktail exposure" to several OPs was associated with increased DNAme aberrancies in sperm. This highlights a particular sensitivity of imprinted genes to preconception environmental exposure, and a non-additive effect of combining multiple exposures, suggesting that broad DNAme measurements should be more systematically considered in such studies. But DNAme is variable in sperm and some maternally derived DNAme loci are less methylated than expected, suggesting that great care is needed in comparative studies. Her ongoing research highlights an exciting new approach in human studies that includes analysis of sperm samples used for IVF and subsequent analysis of fertilized embryos (those not used for implantation). This resembles attempts made in mice and holds promise to unravel mechanistic links between sperm and embryo, a key challenge in basic research.

Pursuing the theme of epigenetic changes induced by chemicals, Marisa Bartolemei (University of Pennsylvania, Philadelphia) presented her work on bisphenol A (BPA) exposure during pregnancy, showing that bile acids and tryptophan metabolism underlie metabolic disease induced in mother and male pups. New results show that these effects can be passed to descendants through the matriline, affecting male offspring. Beyond metabolism, skeletal health also seems to be affected by BPA treatment, which fits with the fact that ovarian hormones play a key role in bone health and points to research needs on the broad effects of chemicals and endocrine disruptors. But although compounds such as BPA, dichlorodiphenyltrichloroethane (DDT) or vinclozolin have long been at the center of studies on epigenetic inheritance, it is still unclear how they, or any environmental factors for that matter, can influence the germline. One possibility is that chemicals can interfere with endogenous factors that can bind directly to receptors expressed in/on germ cells. Indeed, Marine Baptissart (North Carolina State University, Raleigh) presented interesting work from her doctoral studies in the lab of David Volle, showing that a diet fortified with bile acids impacts sperm cells through the G protein-coupled bile acid receptor Tgr5, which is expressed in the germ cell lineage. Her latest data suggest that this effect can be transmitted and impacts DNA condensation due to altered histone/protamine transition, and DNAme in sperm, suggesting that a bile acid receptor in the germline could mediate epigenetic inheritance. To testify the importance of research in the field of epigenetic inheritance in human, Jill Escher (San Jose, CA), a philanthropist and mother of two children with non-verbal autism, presented a moving and personal account linking the tragic story of her family and many other families, with environmental risk factors. Ms Escher urged scientists to study the potential effects of environmental exposure on germ cells *in utero* and the neurodevelopmental outcomes, and presented the activities of her foundation, the Escher Fund for Autism, in support of such research.

Moshe Szyf (McGill University, Montreal) discussed his work on how social stimuli, e.g. licking in rodents, rearing conditions or social rank in primates, and stress affect the epigenome in T cells and germ cells, and stress response in the offspring. He discussed the results of a prospective longitudinal study following the impact of an ice storm in Quebec in 1998, which left millions of Canadians without heat and at winter temperatures. Immune system changes and DNAme levels in T cells of teenage offspring were associated with mother's exposure to the event during pregnancy. Noting that sperm contains glucocorticoid receptors (GR), he hypothesized that GR may be a link between germ cells and the metabolic and psychological uptake of stressful environments, and provided preliminary evidence for this. Other forms of population trauma and their consequences over generations were discussed by Rachel Yehuda (Mount Sinai Hospital, New York). She described the long-term consequences (up to over 50 years) of traumatically stressful experiences such as the Holocaust and 9/11, on survivors and their offspring, and the development of post-traumatic stress disorder (PTSD) and mood or affective disorders. She highlighted sex- and age-specific effects on cortisol levels and sensitivity, associated with changes in the methylation of key genes in related pathways, such as FKBP5, that can be opposite in exposed parents and offspring. In the search for resilience mechanisms, she proposed to "harness epigenetics for positive resilience building."

New Insights and Major Questions Raised by Work in Animal Models

Of all environmental exposures that affect individuals and their offspring, diet is probably one of the most critical and much work has been done on its impact across generations in animals. Building on the notion in humans that the risk for obesity/diabetes can be transmitted, Josep Jimenez-Chillarón (Hospital Sant Joan de Deu, Barcelona) described a mouse model of adiposity based on increased milk intake during postnatal development achieved by reducing litter size. This manipulation leads to metabolic symptoms such as liver-mediated increase in adiposity and altered glucose tolerance, which persist for at least two generations, and are associated with changes in DNAme in sperm of exposed males and liver of the offspring. The metabolic phenotypes appear complex and differ across generations, consistent with the notion of differences between direct and transmitted effects (see below). An enrichment in tRNA fragments (tRFs) was also observed in the testes of exposed males, in line with previous findings that tRFs in sperm are altered by a nutritional insult. In search for underlying mechanisms, Vardhman Rakyan (Blizard Institute, London) presented results on the effects of protein restriction in mice *in utero*, showing a decrease in offspring weight specifically when switching from protein restriction to normal diet after weaning. This suggested here too that the effect results from a mismatch between early and late environment. This was associated with differential methylation and copy number alteration of a DNA locus with a specific genetic variant (A instead of C). Notably, the relative number of A variants determined the degree of DNAme upon exposure to protein restriction, suggesting an important mechanism for genotype–environment interaction.

Considering the long-term consequences of environmental factors on a much larger time scale, Carlos Guerrero-Bosagna (Linköping University) exploited different breeds of domesticated chicken and their only living ancestor, the red jungle fowl, to analyze DNAme. Such analyzes revealed the possibility to accurately reconstruct chicken phylogeny. Preliminary results suggest that, likely through mechanisms of deamination, DNAme may impact the genome by leading to single-nucleotide polymorphisms (SNPs) or copy number variation (CNV). Interestingly, the probability of DNAme correlated with phylogenetic distance, and although the exact causal link is not yet established, these results suggest that environmentally-induced epimutations may drive genomic evolution, a concept he and Michael Skinner already proposed in Darwin finches.

Dating back to the pioneering work of Michael Skinner indeed, endocrine disruptors and chemicals/pollutants have been another key focus of the field of epigenetic inheritance. At the symposium, Skinner gave a broad overview of almost two decades of research from his lab, detailing, cataloging and comparing the transgenerational impact of various compounds on physiological and cellular functions. His latest work comprehensively assessed changes in different epigenetic factors after exposure to the insecticide DDT. He showed that not only DNAme in differentially methylated regions (DMRs), but also short and long non-coding RNAs, and histone retention are altered in sperm after DDT exposure, and are passed to the following generation in a complex pattern, with little direct overlap between individual changes across generations. As one of the main contributors to the field who had long emphasized the important role of DNAme, he summarized the current state of research as follows: "All epigenetic processes are involved in epigenetic inheritance. We need to stop arguing which ones are or aren't involved, they all seem to have different functions." His systematic cross-generational analyzes brought new understanding that beyond epigenetic changes that are directly induced in germ cells of exposed individuals, other changes can emerge in the second or third generation, that do not necessarily match with those in the first generation. This points to a form of epigenetic dynamics across generations. Searching for direct overlap between changes in F1 and F3 might therefore not be that useful and informative.

Regarding the mechanisms underlying epigenetic inheritance, a clear trend in the field is the focus on sperm RNAs as vectors of information transmission from father to offspring. Several speakers presented new data highlighting the involvement of RNA in epigenetic inheritance. Minoo Rassoulzadegan (University of Nice), who has pioneered the field of RNA-mediated epigenetic inheritance, introduced a new concept involving heritable RNAs. She showed how paternal telomere size, usually associated with lifespan, can be inherited. New data from her group suggest that telomeric repeat-containing RNAs (TERRAs), that bind to telomeres and regulate their length, are transmitted from sperm to oocyte upon fertilization. Indeed, telomere size is paternally inherited and associated with paternal lifespan, making these RNAs strong candidates for this transmission.

Using a model of postnatal traumatic stress, Katharina Gapp (Sanger Institute, Cambridge) from the lab of Eric Miska then presented work on the causal involvement of sperm RNAs in epigenetic inheritance. She discussed new results highlighting that short and long non-coding RNAs must be considered together to understand their role in information transfer from sperm to oocyte/offspring. She showed that injection of long RNAs from the sperm of males exposed to traumatic stress in postnatal life into wild-type zygotes can recapitulate some of the phenotypes observed in directly exposed males, but short RNAs cannot or produce different effects, suggesting that long RNAs are also involved in transmission. A following talk on the same mouse model addressed the question of the potential vectors that alter the epigenome in germ cells. Before any substance can reach the germline and modify its epigenome, it needs to get to the testis and/or epididymis. This transport may occur through the bloodstream. Gretchen van Steenwyk from the lab of Isabelle Mansuy (University/ETH Zürich) showed that blood-borne factors are causally linked to the inheritance of phenotypes induced by postnatal trauma. Blood therefore appears as an important biological fluid, and highly relevant for translational approaches since it is easily accessible in humans.

Further to humans and mice, several other animal models also provided mechanistic insight into epigenetic inheritance. Abhay Sharma (Delhi University) reported the transgenerational impact of high sugar diet on body weight and chromatin remodeling in *Drosophila*. Notably, an opposite phenotype is observed in F1 and F2, and the number and identity of affected genes are also different between females and males. A switching experimental design highlighted that it is not so much the diet itself, but rather the mismatch between parental and offspring diet that causes metabolic alterations. Eric Miska (University of Cambridge) discussed epigenetic inheritance in honey bees, an interesting model system in which epigenetic mechanisms play a key role in the development of different casts of genetically identical individuals. He presented intriguing data on another unusual feature of honey bees, which is the ability to use a double-stranded RNA that can be stored in royal jelly, bees' food source, and serve to protect against a deadly virus. RNA sequencing data revealed that royal jelly carries both, bee and viral RNA bound by a yet unknown protein termed "royal protein" that stabilizes them and acts as a transfection agent allowing RNA to be transported into cells. Such system could be used to feed bees, and potentially other species, with RNAi protecting them against infection. Finally, Leah Houri-Ze'evi (Tel Aviv University) from the lab of Oded Rechavi presented elegant work on *C. elegans*, a working horse in epigenetic inheritance research with a powerful associated genetic toolbox. She demonstrated that the extent of environmentally-triggered epigenetic inheritance is constrained by an active "forgetting" process. This argues against a "dilution model," which has often been used to explain why RNAi-based inheritance in *C. elegans* typically fades away after four generations.

Methodologies in Epigenetics

Methodological and computational challenges of epigenomic studies were extensively addressed during the symposium. Jorg Tost (Commissariat à l'Energie Atomique, Evry) discussed the use of tools such as CRISPR-dCas9 for epigenetic editing and the possibility to intervene on the epigenetic profile of specific loci. Such approach can circumvent a long-standing caveat of molecular epigenetics which has been its reliance on correlation, and now offers means for causality. He highlighted, however, the difficulties in inducing precise, effective and stable changes, depending on vectors, tissues and sites used, and discussed the potential of this rapidly developing tool. While such approaches are expected to help functional validation in epigenetic studies, more work is needed to improve them and streamline their application. To help tame the complexity and amount of epigenetic data collected by new methodologies, Christoph Bock (CeMM of the Austrian Academy of Sciences, Vienna) presented a series of computational tools and approaches for epigenomics, in particular for the study of DNAme in cancer. He illustrated how epigenome profiling can be used to track the origin of cancers of unknown primary site, or for biomarker development. He also highlighted recent progress on single cell multi-omics analyzes, and provided several important advice for planning and conducting data analyzes, and for epigenetic data interpretation.

Following on technologies, Stephan Beck (University College London) discussed a series of issues, solutions and insight in epigenome-wide association studies, from statistical analysis to functional validation, and future perspectives for epigenome profiling in humans. He also harnessed the accessibility of blood in human cohorts, measuring for instance biological aging

from a DNAme mitotic clock in the UK's Personal Genome Project. This concluded that while the link between bench and bedside in epigenetic inheritance is still in its infancy, basic epigenetics research in general is making major strides toward informing diagnosis and potential treatment in humans. Finally, working on Alzheimer's disease (AD), Johannes Graeff (Ecole Polytechnique de Lausanne) showed how specific methods, in this case, chromatin conformation capture (CCC), can help gain insight into the intimate mechanisms of diseases. CCC was used to identify the target of a methylation quantitative trait locus (mQTL) initially flagged by its flanking SNPs found to be associated with AD and responsible for mQTL epigenetic status (DNAme). He demonstrated that the identified target is indeed relevant for AD because its upregulation promotes survival and reduces amyloid-ß plaques, a hallmark of AD pathology. This extended the relevance of links between DNAme and genetic variants, and the potential consequences on 3D chromatin structure.

Evolution, Societal Impact and Broader Considerations

The final session of the symposium sought to put epigenetic inheritance into a broader evolutionary and societal context. It was opened by Eva Jablonka (Tel Aviv University), who offered a theoretical view connecting inheritance and associative learning, highlighting the risk of overlearning inherent to both, and therefore the importance of mechanisms of forgetting. Such plasticity is ensured by mechanisms of epigenetic inheritance, which are highly advantageous in shifting environments. She further developed this hypothesis in her discussion of the evolution of both associative learning and epigenetic inheritance, ultimately merging them onto the broader concept of learning and memory. She argued that, along with other factors, the evolution of associative learning can help explain the rapid divergence during the Cambrian explosion. In this narrative, the evolution of learning in a rapidly changing environment presented the threat of *overlearning*, and in this context it was critical for organisms to evolve mechanisms of active forgetting that would ensure sufficient plasticity. Transgenerational epigenetic inheritance is therefore to be expected as highly adaptive. Liran Carmel (Hebrew University of Jerusalem) followed up by talking about the profiling of ancient epigenome from bone remnants and presented ways to reconstruct DNAme patterns in archaic humans, in particular, Neanderthal. He showed that DMRs have appeared during evolution in Neanderthal, Denisovan and modern human, and that genes identified in some of these DMRs are relevant for skeletal development for instance of larynx, an organ important for speech. He explained how DNAme can be inferred from ancient DNA sequencing, and help identify methylation changes in early humans, potentially enabling the reconstructed DNAme profiles to predict ancient environmental exposure.

The last two talks finally focused on societal aspects of epigenetic inheritance. Maurizio Meloni (University of Sheffield) showed that plasticity has an old history only shadowed by twentieth century genetics, and that there is a massive imbalance in scientific studies toward the identification of epigenetic factors underlying negative or deleterious features, which suggests a bias in the questions asked. Ruth Müller (Technical University of Munich) then discussed critical issues in the way researchers frame the problem they investigate and communicate their results. For instance, studies showing the transmission of epigenetic marks associated with poverty can be reported in ways that stigmatize the poor and gives a feeling of fatality, which is negative. However, such results can also be mitigated by systematically emphasizing the plasticity or reversibility of epigenetic marks and thus send a more positive message to society. It is therefore critical to be aware of the social context of the problems under study, and the importance of properly evaluating the medical, societal and moral impact of the concept and any related new discovery in epigenetics.

Conclusions

Epigenetic inheritance has emerged as an extremely exciting field of research, with novel and often alternative concepts, numerous study models and highly innovative methodologies that are constantly and rapidly evolving. The symposium was particularly enlightening in putting in perspective the current hypotheses and evidence in different experimental models and humans, and in discussing current conceptual and technical challenges. It opened important debates and necessary reflection about theories, experimental modeling, bioinformatics, and in the design of epidemiological, ethological, ethical and human studies that are expected to promote research in the field in the upcoming decades.

Isabelle Mansuy

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Further to the Symposium

Poster Session

There were two poster sessions included in the program, during which 20 posters were presented by young scientists.

These sessions were extremely well attended and a prize was awarded to one poster at the end of the symposium. The winning poster was entitled "One sperm for all—a novel experimental strategy opens new horizons in epigenetic inheritance" and was presented by Martin Roszkowski, PhD student at the Brain Research Institute, Zürich.

LS2 Workshop "Meet the Experts"

In addition to the Latsis symposium, a satellite workshop (supported by Life Science Switzerland LS2) was organized at the University of Zürich following the symposium. This workshop entitled "Meet the Experts" had four experts in the field of epigenetics, Eva Jablonka, Isabelle Mansuy, Michael Skinner and Moshe Szyf answering questions from the audience. The workshop was attended by 30 people.

Public Lecture

A public lecture in German entitled "Erbe, Umwelt und Vergangenheit: Warum die Epigenetik den Blick auf die Gesundheit verändert" (Heritage, environment and past: why epigenetics changes the view on health) was presented by a scientific writer and journalist, Dr Peter Spork. This public lecture was intended for the lay audience and discussed the impact of the field on the society with a reflection on major societal questions. It was followed by an autograph session during which the public could talk to Peter Spork and get his bestseller book "Gesundheit ist kein Zufall" (Health is not by chance).

Conflict of interest statement. None declared.